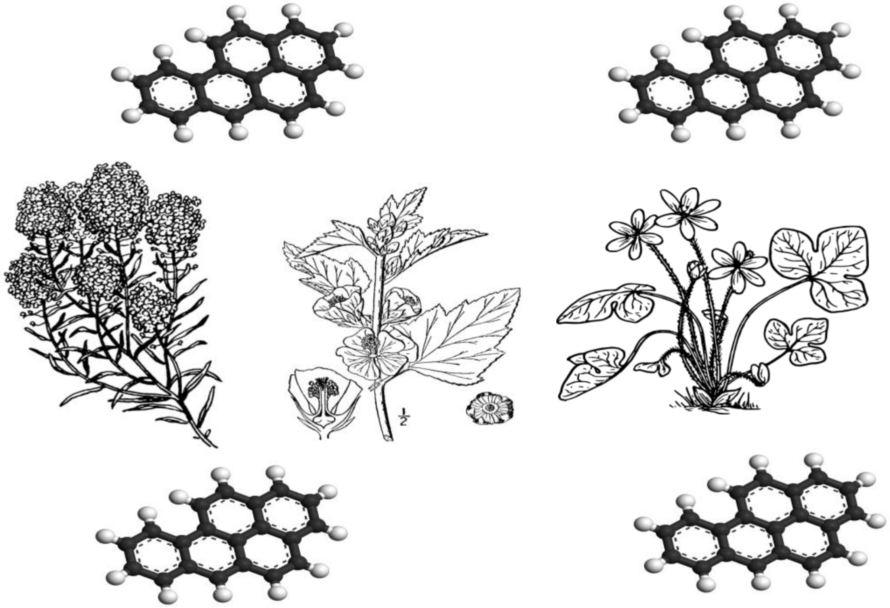


indena

INDUSTRIA
DERIVATI
NATURALI

SANA BOLOGNA 2018
Botanicals and contaminants: emerging issues or new managing of existing risks?

IPA (Idrocarburi Policiclici Aromatici) NELLE PIANTE PER USO ALIMENTARE

PAH (Polyciclic Aromatic Hydrocarbons) in plants for food use

R. Iguera, E.M. Martinelli

indena

PAHs – DEFINITION

Polycyclic aromatic hydrocarbons (PAHs) form a large group of over 200 different chemicals containing two or more fused aromatic rings made up of carbon and hydrogen atoms.

The most abundant PAHs in the environment contain between two and seven rings.

efsa
European Food Safety Authority

The EFSA Journal (2008) 724, 1-114

Polycyclic Aromatic Hydrocarbons in Food[1]

Scientific Opinion of the Panel on Contaminants in the Food Chain

(Question N° EFSA-Q-2007-136)

Adopted on 9 June 2008

PAHs – CHEMICAL STRUCTURES

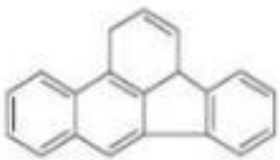
Some Polycyclic Aromatic Hydrocarbons (which originate from combustion processes) are carcirogenic and mutagens

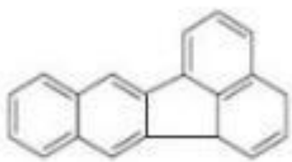

benzo(a)anthracene ($C_{18}H_{12}$)


chrysene ($C_{18}H_{12}$)


5-methyl chrysene ($C_{19}H_{14}$)

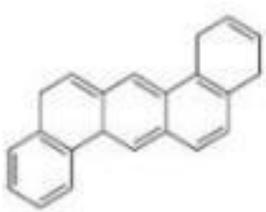

benzo(j)fluoranthene ($C_{20}H_{12}$)


benzo(b)fluoranthene ($C_{20}H_{12}$)


benzo(k)fluoranthene ($C_{20}H_{12}$)


benzo(a)pyrene ($C_{20}H_{12}$)


dibenzo(al)pyrene ($C_{24}H_{14}$)


dibenzo(ah)anthracene ($C_{22}H_{14}$)


dibenzo(ae)pyrene ($C_{24}H_{14}$)


dibenzo(ai)pyrene ($C_{24}H_{14}$)


dibenzo(ah)pyrene ($C_{24}H_{14}$)


indeno 123 cd-pyrene ($C_{22}H_{12}$)

indena®

PAHs – MECANISM FORMATION

Majority of the PAHs in the environment are derived from reaction at high temperature during pyrolytic condition with incomplete combustion

PYROLYSIS
Complex organic molecules are partially cracked to smaller unstable fragments, mainly active free radicals with very short average lifetime

↓

PYROSINTHESIS
These fragments are unstable and partly combusted and partly recombined to form stabile PAHs

NATURAL AND ANTHROPOGENIC SOURCES

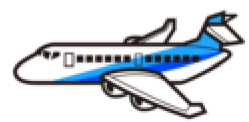
NATURAL

FIRE

VOLCANOS

ANTHROPOGENIC

VEHICLES



AIRPLAN

INDUSTRIAL PROCESS

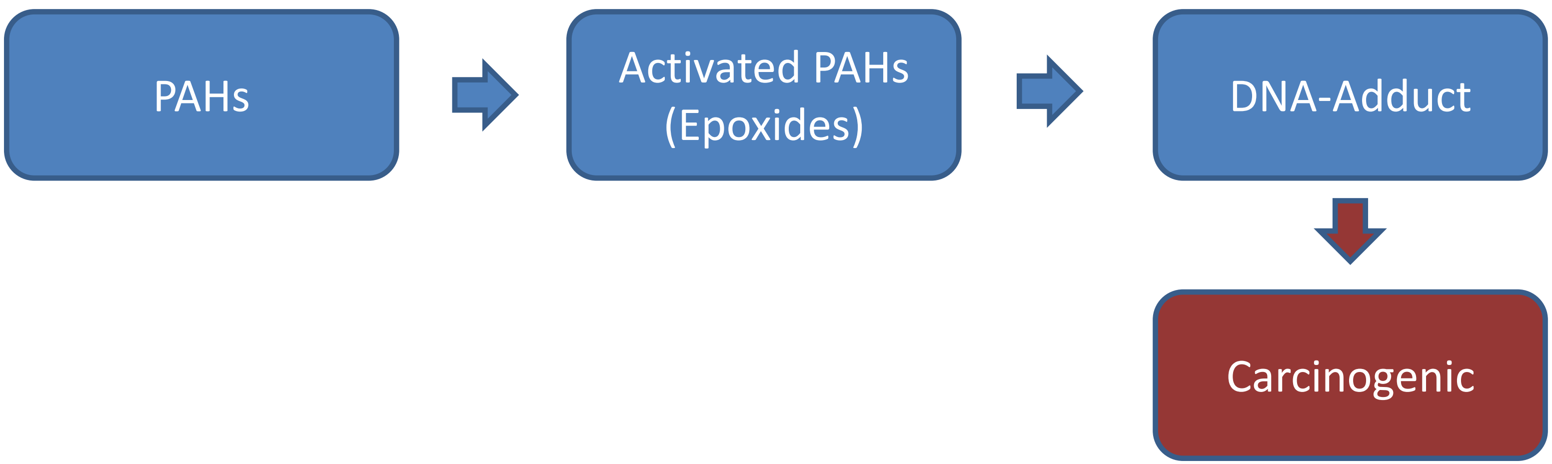
WOOD BURNING

VEHICLES

PAHs: Carcinogenicity and Genotoxicity

- 2002 SCF (Scientific Committee on Food) concluded that 15 PAHs show clear evidence of mutagenity/genotoxicity.

- 2005 JECFA (Joint FAO/WHO Expert Committee on Food Additives) concluded that 13 PAHs was clearly genotoxic and carcinogenic.



indena®

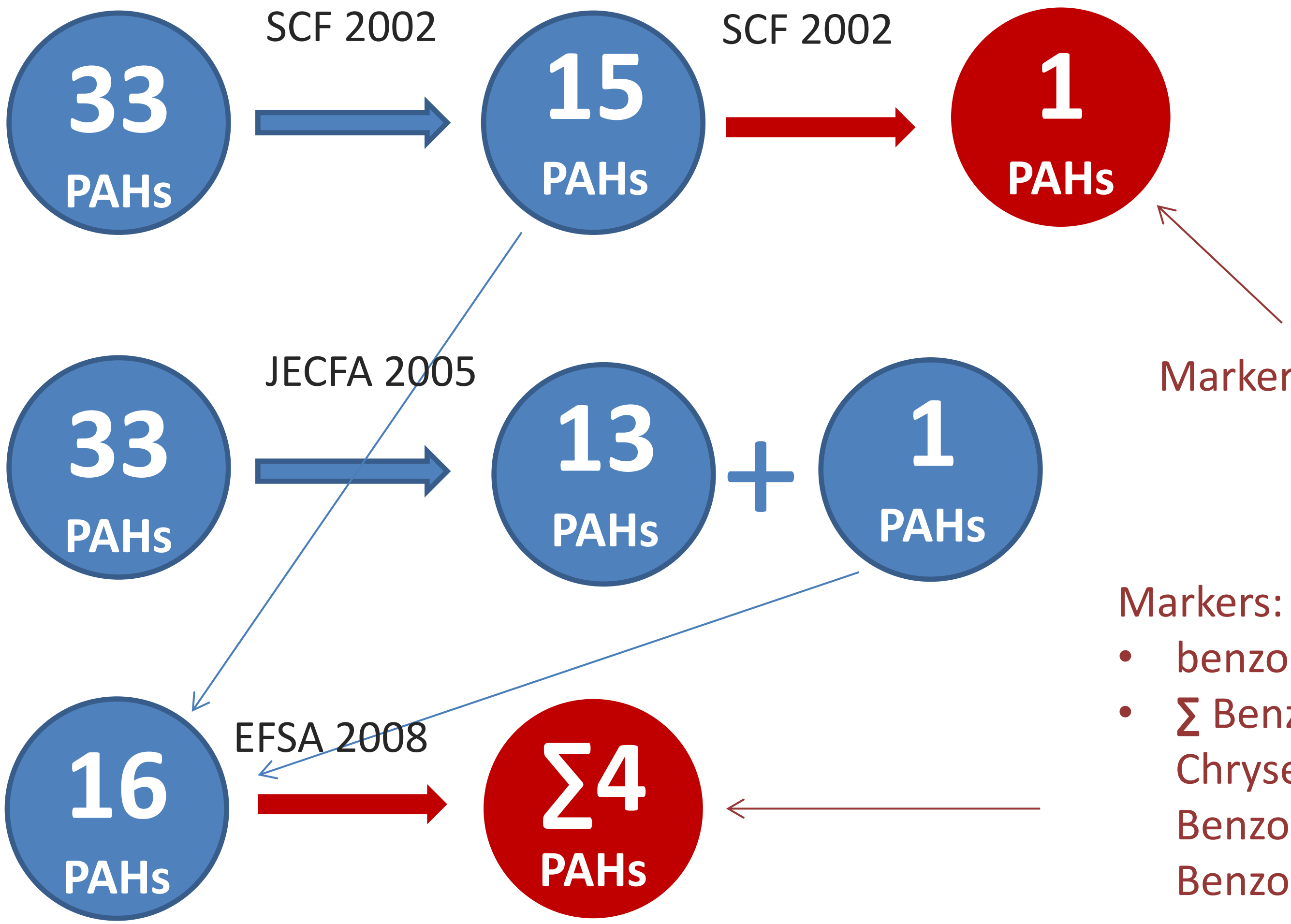
PAHs: Carcinogenicity and Genotoxicity

In humans, the major routes of uptake of PAH are thought to be through

1. **the gastro-intestinal tract after ingestion of contaminated food or water;**
2. the lungs and the respiratory tract after inhalation of PAH-containing aerosols or particulates to which a PAH, in the solid state;
3. the skin as a result of contact with PAH-bearing materials.

indena®

WHICH PAHs WE NEED TO CONSIDER?



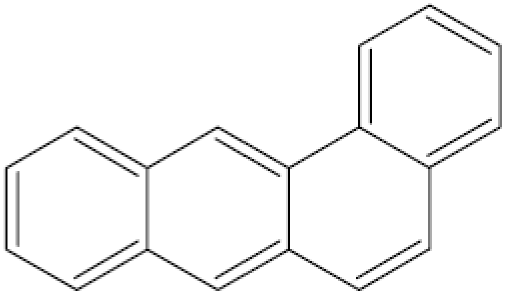
Marker; benzo[a]pyrene

Markers:
- benzo[a]pyrene
- ∑ Benzo[a]pyrene; Chrysene; Benzo[a]anthracene; Benzo[b]fluoranthene

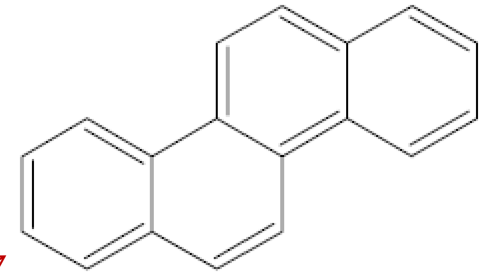
SCF 2002 : http://ec.europe.eu/food/fs/scf/out153_en.pdf
JEFCA 2005: http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf
EFSA 2008: The EFSA Journal (2008) 724, 1-114

indena®

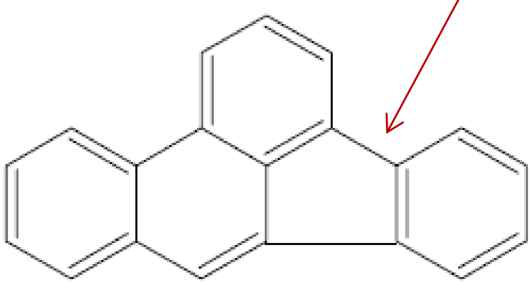
PAH4: Physical and Chemical properties

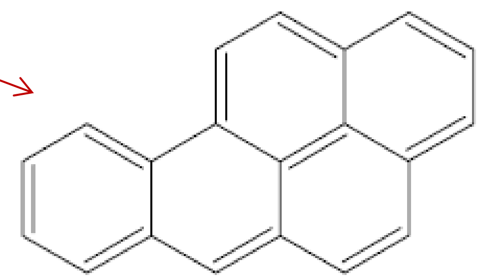


Lipophilic properties



PAH	Abbreviation	Number of rings	MW	Solubility in water mg/L (25 °C)	Octanol-water partition coefficient (log Kow)
Benzo[a]anthracene	BaA	5	228.3	0.0094	5.76
Chrysene	CHRY	5	228,3	0.002	5.73
Benzo[a]pyrene	BaP	6	252.3	0.00162	6.13
Benzo[b]fluoranthene	BbF	6	252.3	0.0015	5.78





MAIN CONTAMINATION PATHWAYS FOR HERBS

1

PRE HARVEST / PRE COLLECTION
= UPTAKE DURING THE GROW OF THE PLANTS

2

POST HARVEST/POST COLLECTION
= CONTAMINATION DURING THE PROCESS

indena®

UPTAKE PATHWAYS - LITERATURE

1. *Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation* - S. Khan et al. / Journal of Hazardous Materials 152 (**2008**) 506–515
2. *Dry deposition of atmospheric polycyclic aromatic hydrocarbons in three plantago species.* - M.I. Bakker et al.Environ. Toxicol. Chem. 18, **1999**
3. *Polycyclic aromatic hydrocarbons (PAHs) pollution recorded in annual rings of gingko (Gingko biloba L.): Determination of PAHs by GC/MS after accelerated solvent extraction.* - H. Yin et al. / Microchemical Journal 97 (**2011**) 138–143
4. *Bioconcentration of polycyclic aromatic hydrocarbons in vegetables grown in an industrial area* - A.M. Kipopoulou et al. / Environmental Pollution 106 (**1999**) 369±380
5. *Polycyclic aromatic hydrocarbons in leaf cuticles and inner tissues of six species of trees in urban Beijing* - Y.Q. Wang et al. / Environmental Pollution 151 (**2008**) 158e164
6. *Uptake of polycyclic aromatic hydrocarbons by maize plants* - H. Lin et al. / Environmental Pollution 148 (2007) 614e619
7. *PAHs associated with the leaves of three deciduous tree species. II: uptake during a growing season* - M. Howsam et all. / Chemosphere 44 (**2001**) 155-164.
8. *Polycyclic aromatic hydrocarbons in agricultural soils in Poland: preliminary proposals for criteria to evaluate the level of soil contamination* – B. Maliszewska – Kordybach applied geochemistry vol. 11 pp. 121-177, **1996**
9. *Polycyclic aromatic hydrocarbons content in shoots and leaves of willow(salix viminalis) cultivated on the sewage sludge-amended soil* - P. OLESZCZUK AND S. BARAN Water, Air, and Soil Pollution (**2005**) 168: 91–111
10. *Soil-to-Root Transfer and Translocation of Polycyclic Aromatic Hydrocarbons by Vegetables Grown on Industrial Contaminated Soils*S - FISMES ET AL J. ENVIRON. QUAL., VOL. 31, SEPTEMBER–OCTOBER **2002**.
11. *An analysis of soil and plant (taraxacum officinale) contaminationwith heavy metals and polycyclic aromatic hydrocarbons (pahs) in the area of the railway junction iława główna, polan* - M. MALAWSKA AND B. WIŁKOMIRSKI *Water, Air, and Soil Pollution* **127:** 339–349, **2001**.
12. *Uptake and Acropetal Translocation of Polycyclic Aromatic Hydrocarbons by Wheat (Triticum aestivum L.) Grown in Field-Contaminated Soil* - Yu q i a n g t a o et all. VOL. 43, NO. 10, **2009** / ENVIRONMENTAL SCIENCE & TECHNOLOGY
13. *Uptake Pathways of Polycyclic Aromatic Hydrocarbons in White Clover* - Ya n z h e n g g a o † a n d C . D. C o l l i n s ENVIRONMENTAL SCIENCE & TECHNOLOGY / VOL. 43, NO. 16, **2009**

UPTAKE PATHWAYS – LITERATURE

Possible pathways

1. Gaseous deposition to leaves via cuticle or via stomata followed by subsequent translocation by sap and water flow.

2. Dry deposition of particles-bound via foliage.

3. Passive and active uptake soil-to-root, followed by subsequent translocation by the transpiration stream, root-to-shoot.

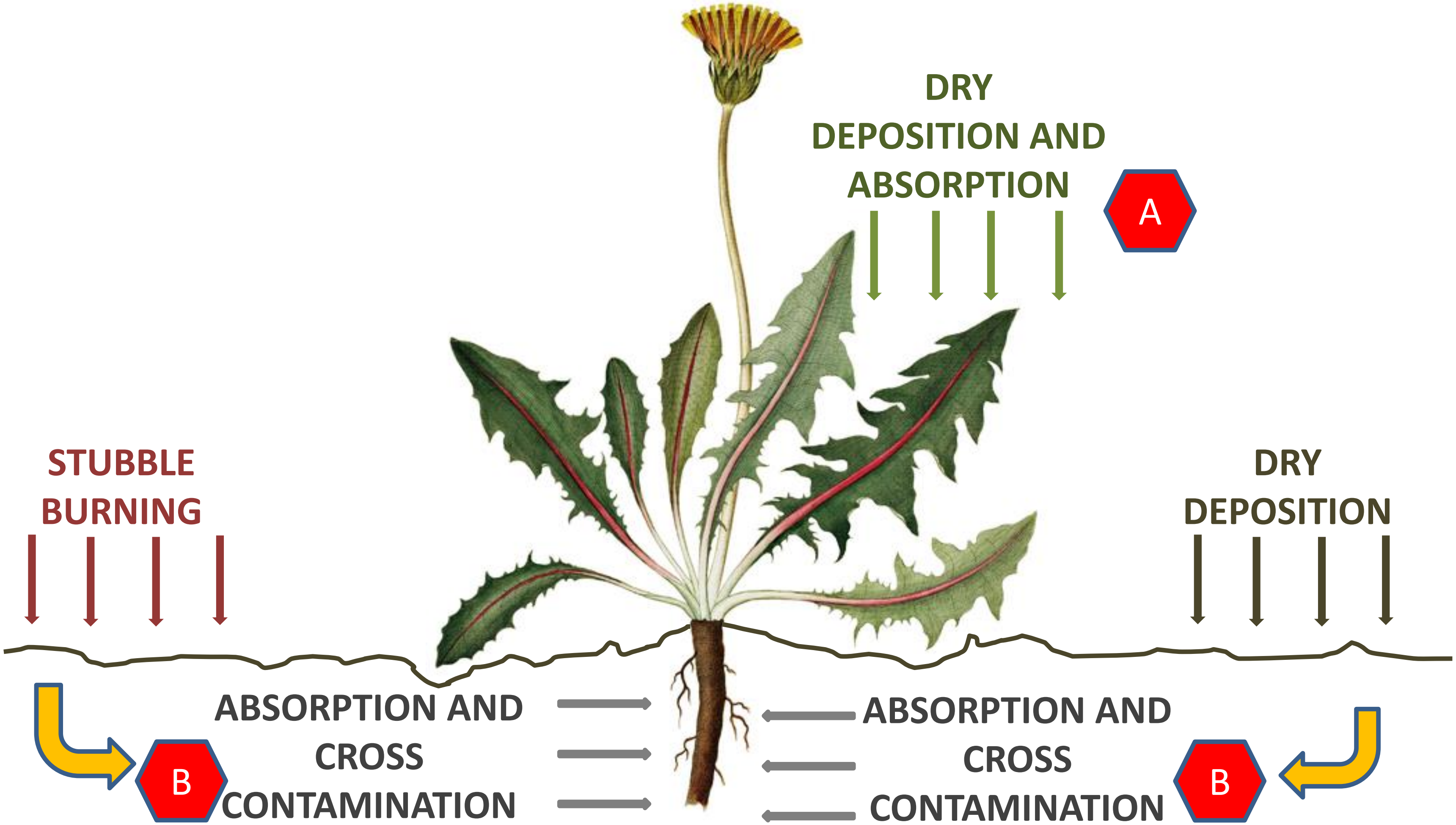
UPTAKE PATHWAYS – LITERATURE

Plant uptake factors

Plant uptake of PAHs varies significantly and is affected by several factors including:

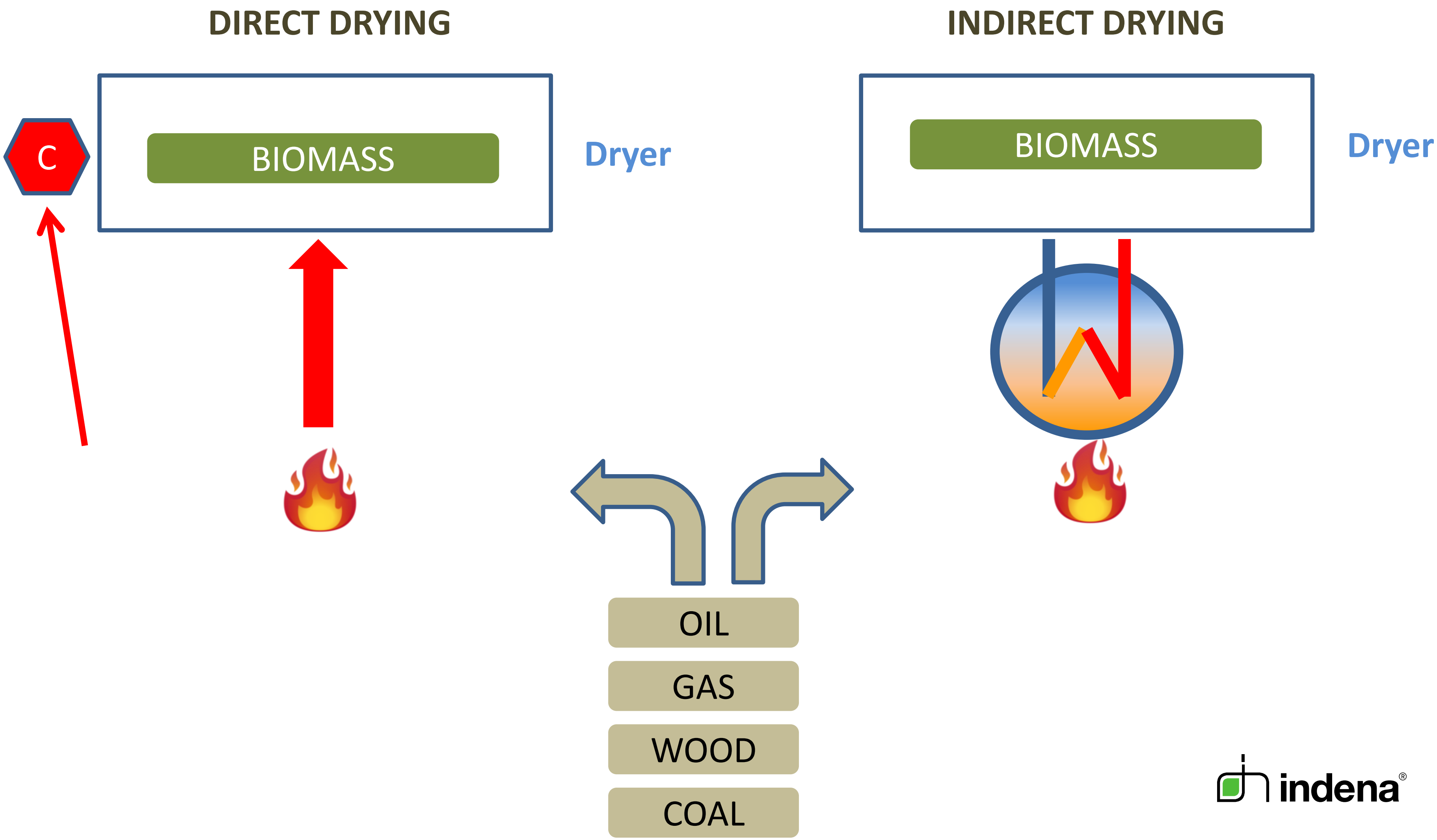
1. **Soil and air concentration**
2. **Plant species**

CONTAMINATION OF HERBS (Botanical)
1 - In the field – uptake pathways



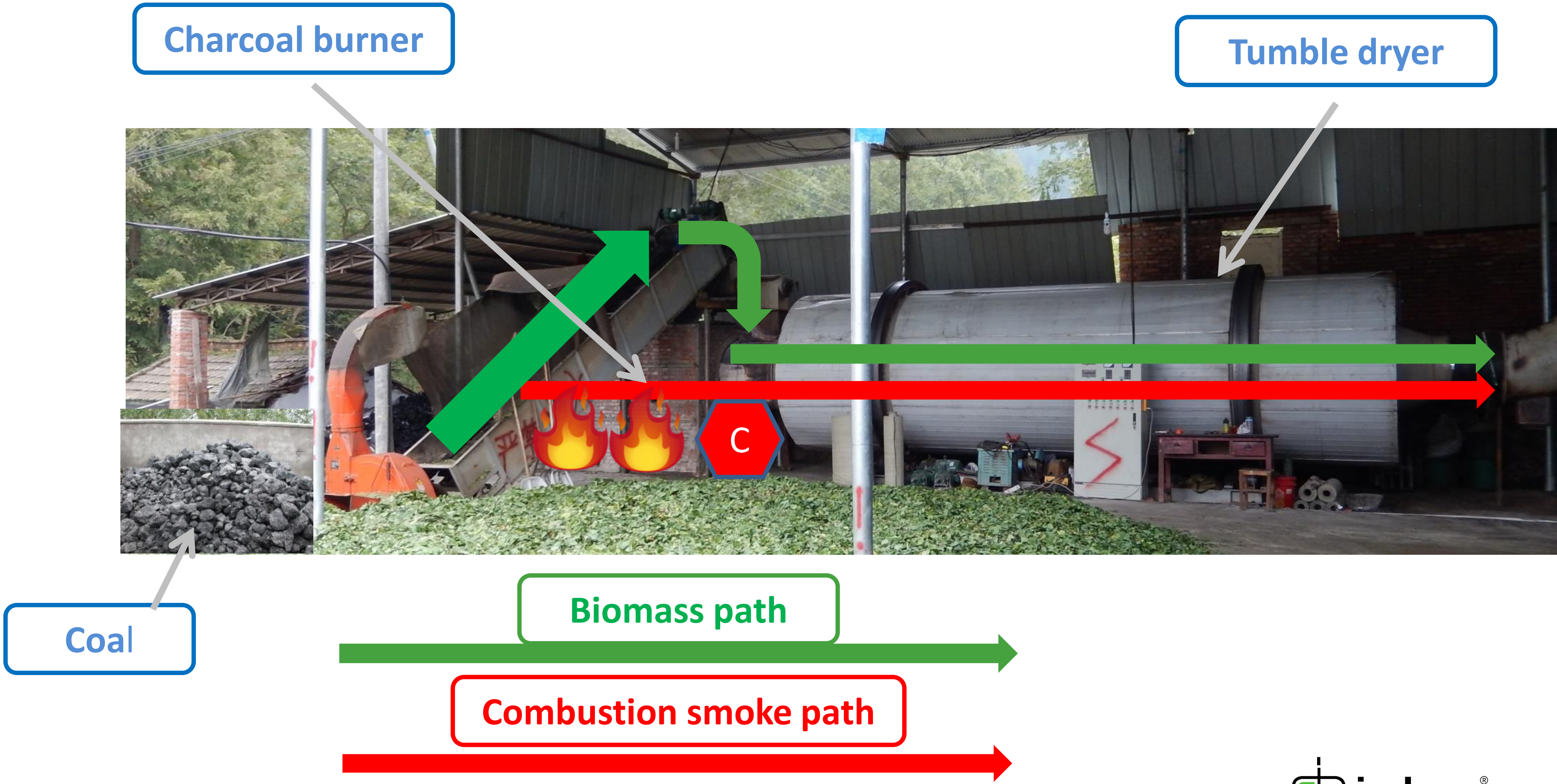
CONTAMINATION OF HERBS

2 - During the drying process - contamination



indena®

CONTAMINATION OF HERBS
During the drying process – direct drying

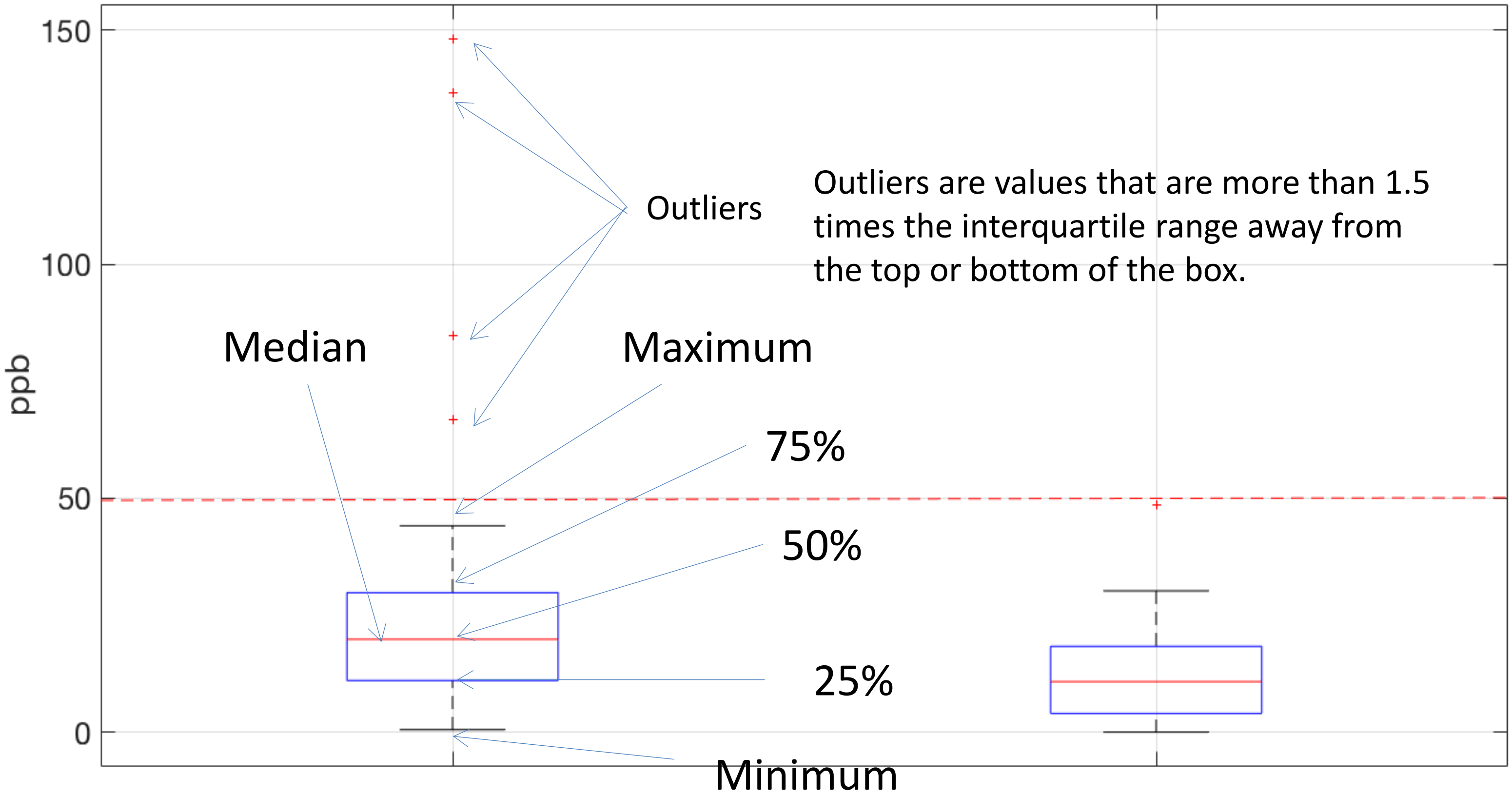


PARTS OF THE PLANT: impact on PAH4

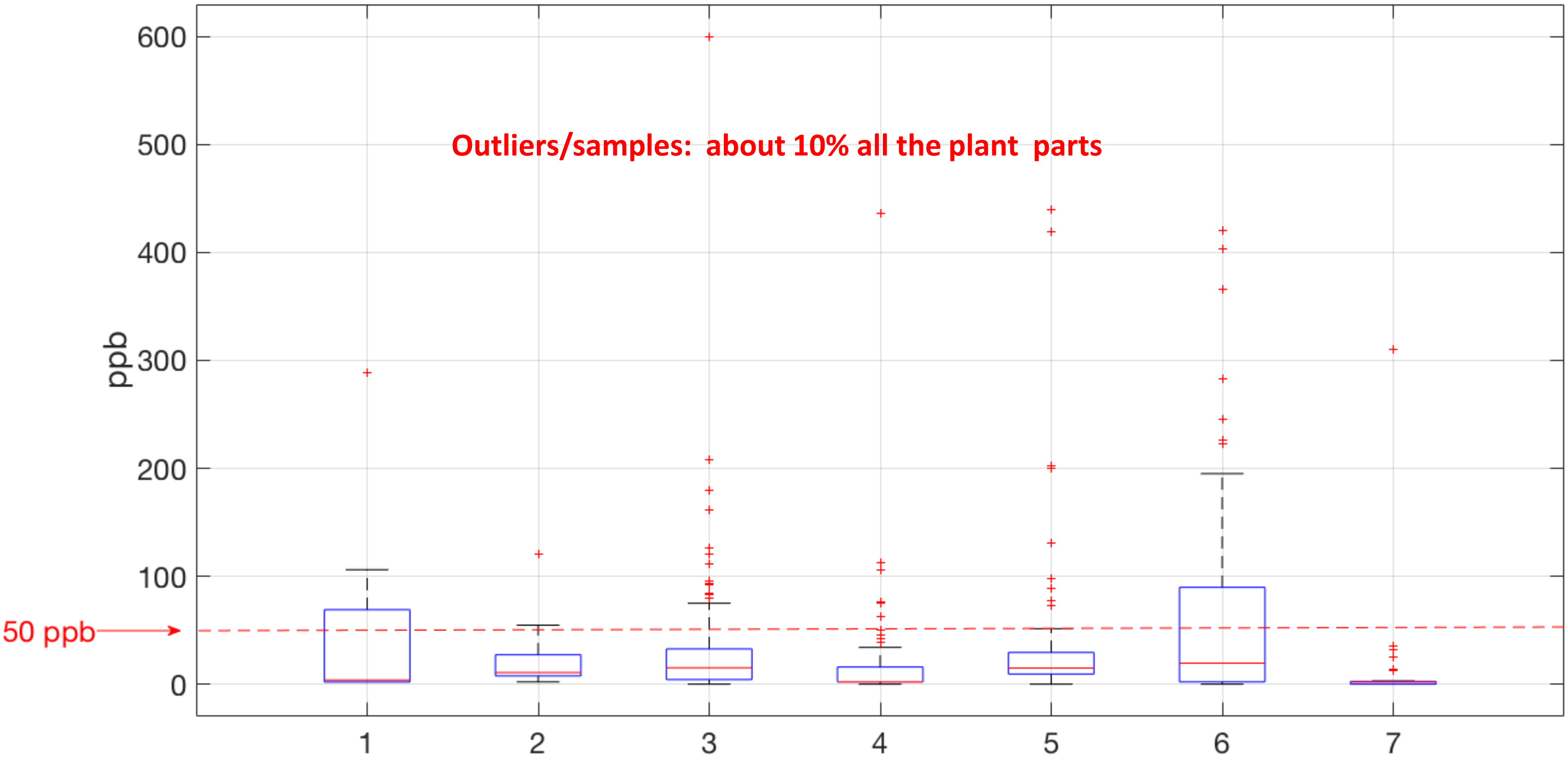
Part of the plant	Number of samples	Number of Species
BARK	10	8
FLOWER	13	9
LEAF	119	34
FRUIT	77	54
HERBA	98	37
ROOT/RHIZOME	79	29
SEED	31	20

Data provided by EUROPAM

BOX PLOT STATISTICS

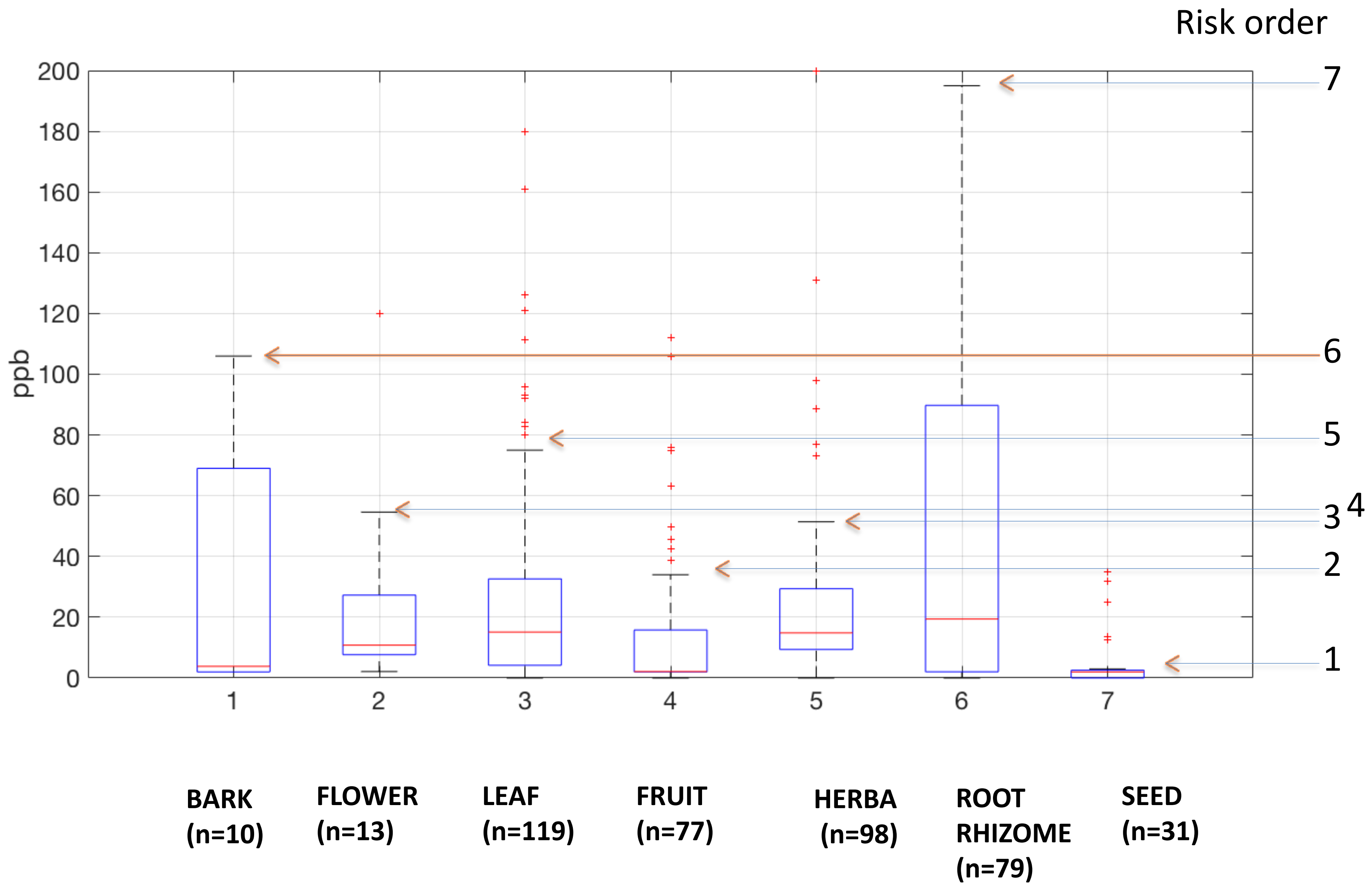


PARTS OF THE PLANT: impact on PAH4



BARK (n=10)	FLOWER (n=13)	LEAF (n=119)	FRUIT (n=77)	HERBA (n=98)	ROOT RHIZOME (n=79)	SEED (n=31)

PARTS OF THE PLANT: impact on PAH4



PARTS OF THE PLANT: impact on PAH4

Part of the plant	Number of samples	Number of Species	Risk order
ROOT/RHIZOME	80	29	7
BARK	10	8	6
LEAF	120	34	5
FLOWER	13	9	4
HERBA	99	37	3
FRUIT	79	54	2
SEED	31	20	1

ORIGINS OF THE HERBS: impact on PAH4

Valeriana officinalis rhizomes and roots from Europe

indena®

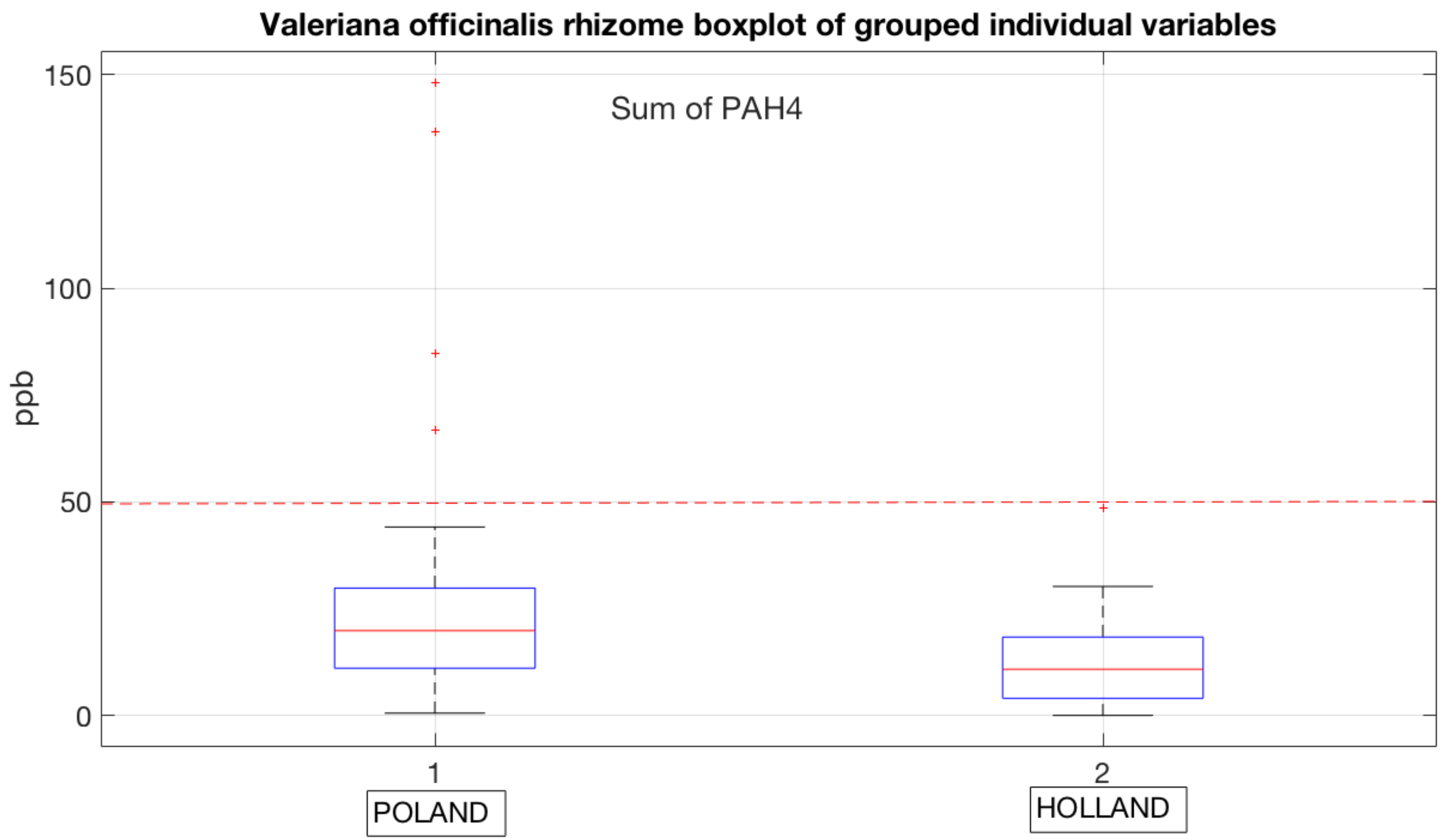
ORIGINS OF THE HERBS: impact on PAH4

Valeriana officinalis rhizomes and roots from Europe

Origin	Samples n.	Drying
Poland (2 suppliers)	28	Indirect
Holland (2 suppliers)	29	Indirect

ORIGINS OF THE HERBS: impact on PAH4

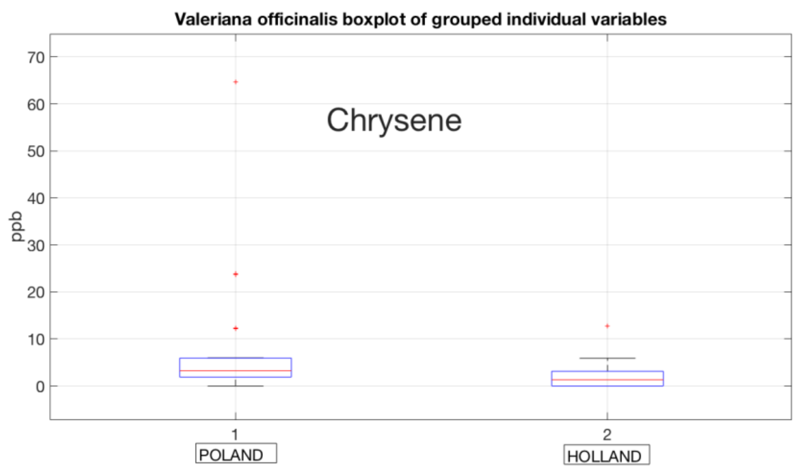
Valeriana officinalis rhizomes and roots from Europe

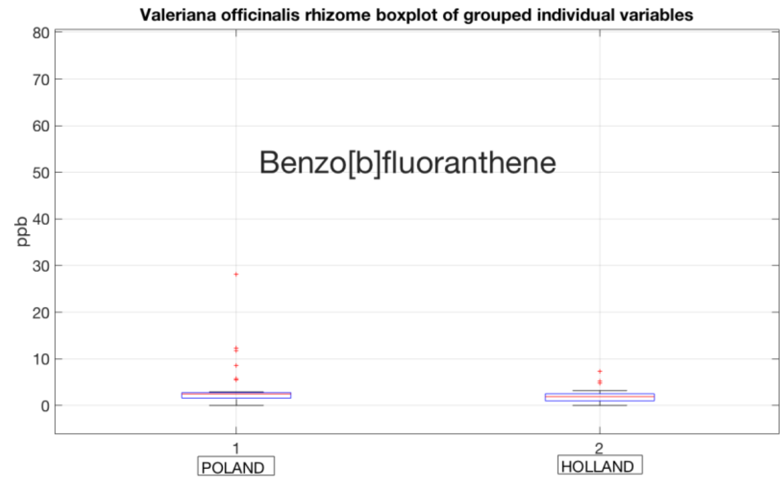


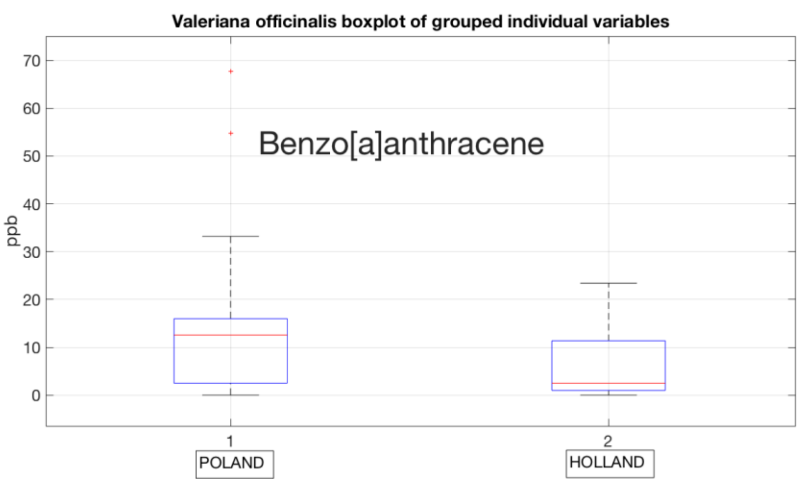
indena®

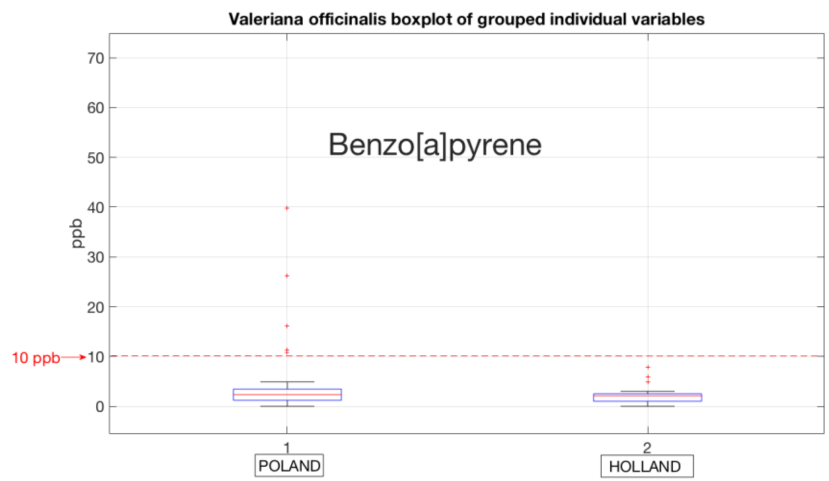
ORIGINS OF THE HERBS: impact on PAH4

Valeriana officinalis rhizomes and roots from Europe





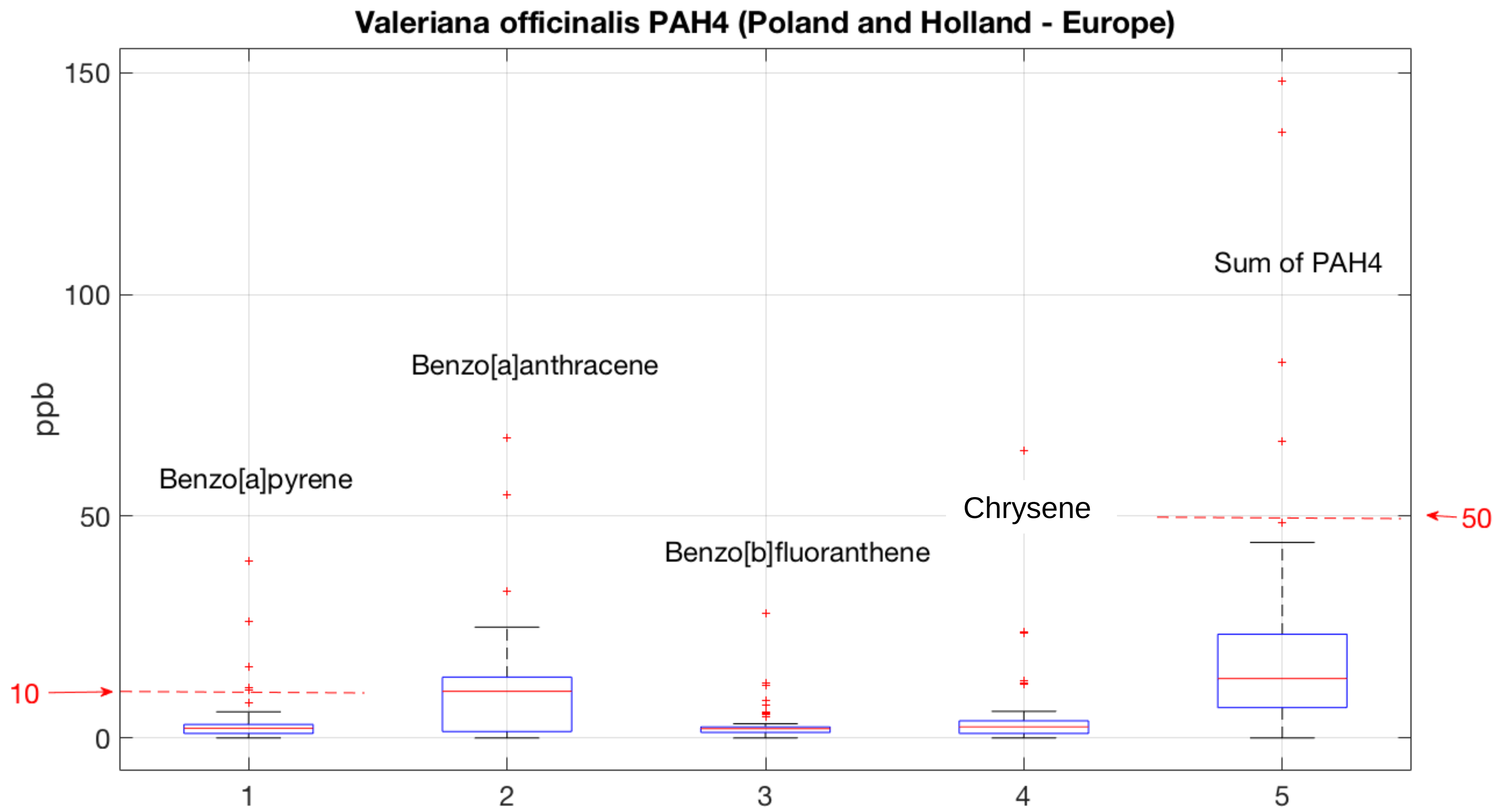




indena®

ORIGINS OF THE HERBS: impact on PAH4

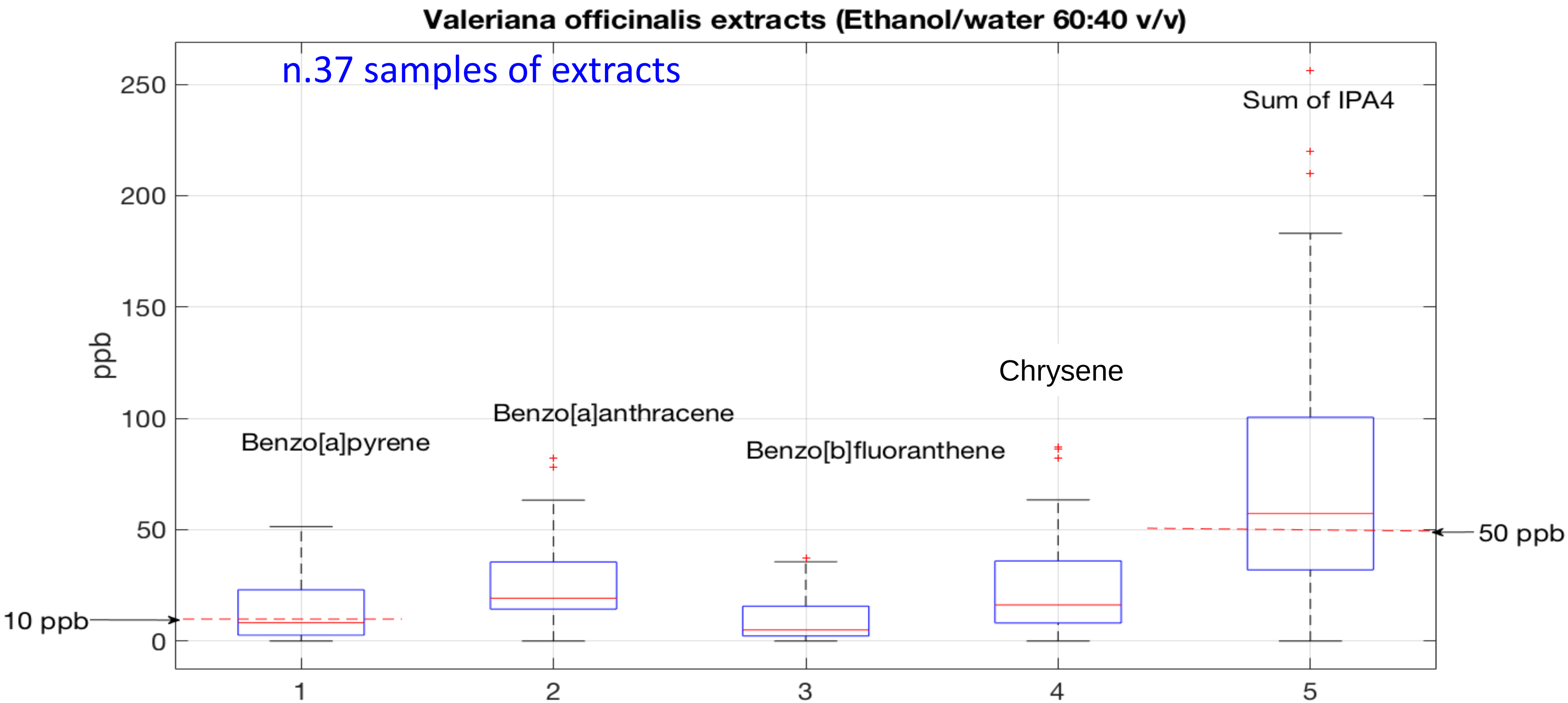
Valeriana officinalis rhizomes and roots from Europe



indena®

ORIGINS OF THE HERBS: impact on PAH4

Valeriana officinalis rhizomes and roots **extracts** (Ethanol/Water)



ORIGINS OF THE HERBS: impact on PAH4

Valeriana officinalis – Risk evaluation for PAHs

HERBS - RHIZOME AND ROOTS

B

Passive and active uptake soil-to-root

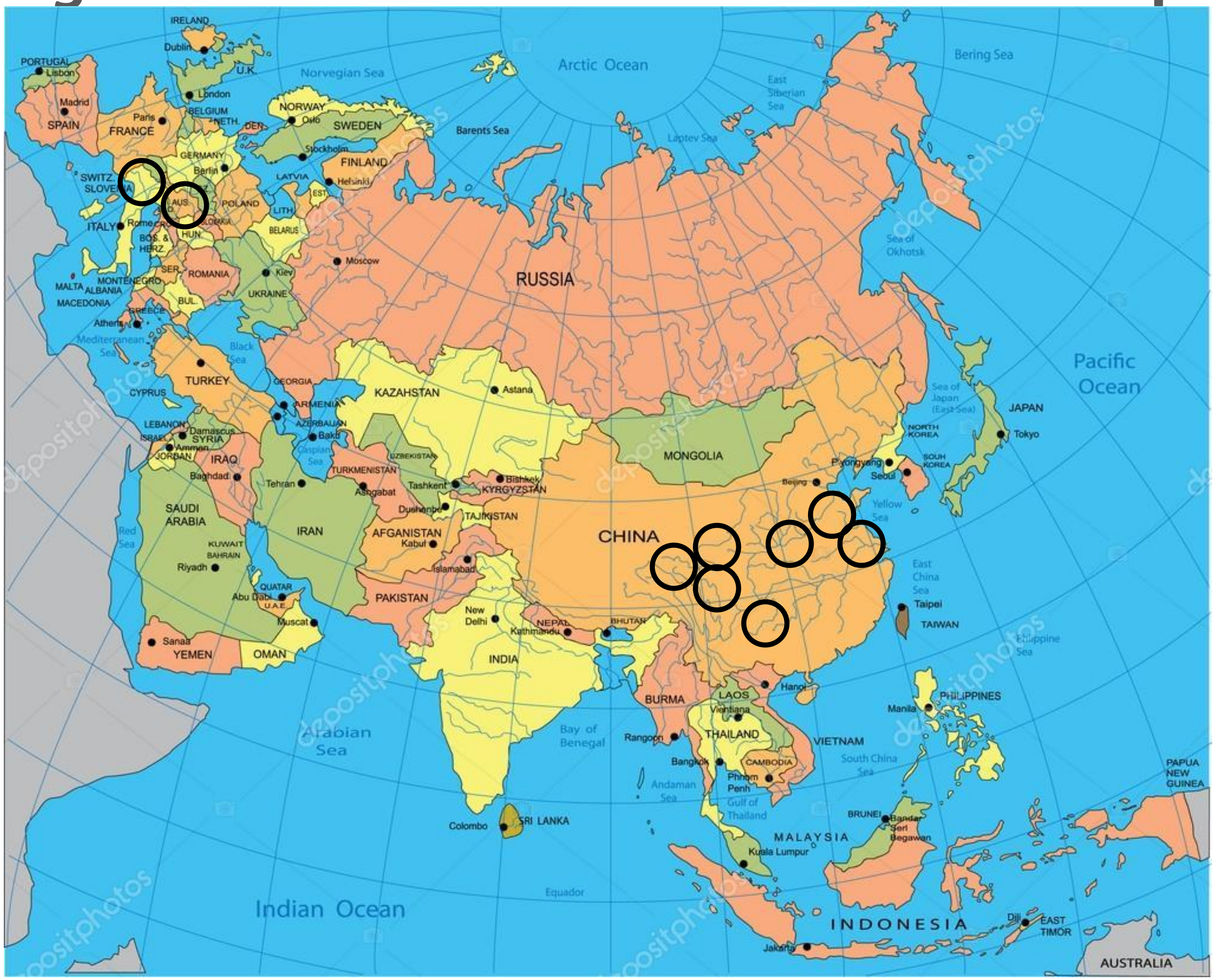
Cross - contamination with soil residue

BOTANICAL EXTRACT

- **Risk carry-over of PAHs** due to solvent extraction (total extract, without liphophilic purification)
- **Risk of concentration effect (DER)**

ORIGINS OF THE HERBS: impact on PAH4

Ginkgo biloba leaves from China and Europe



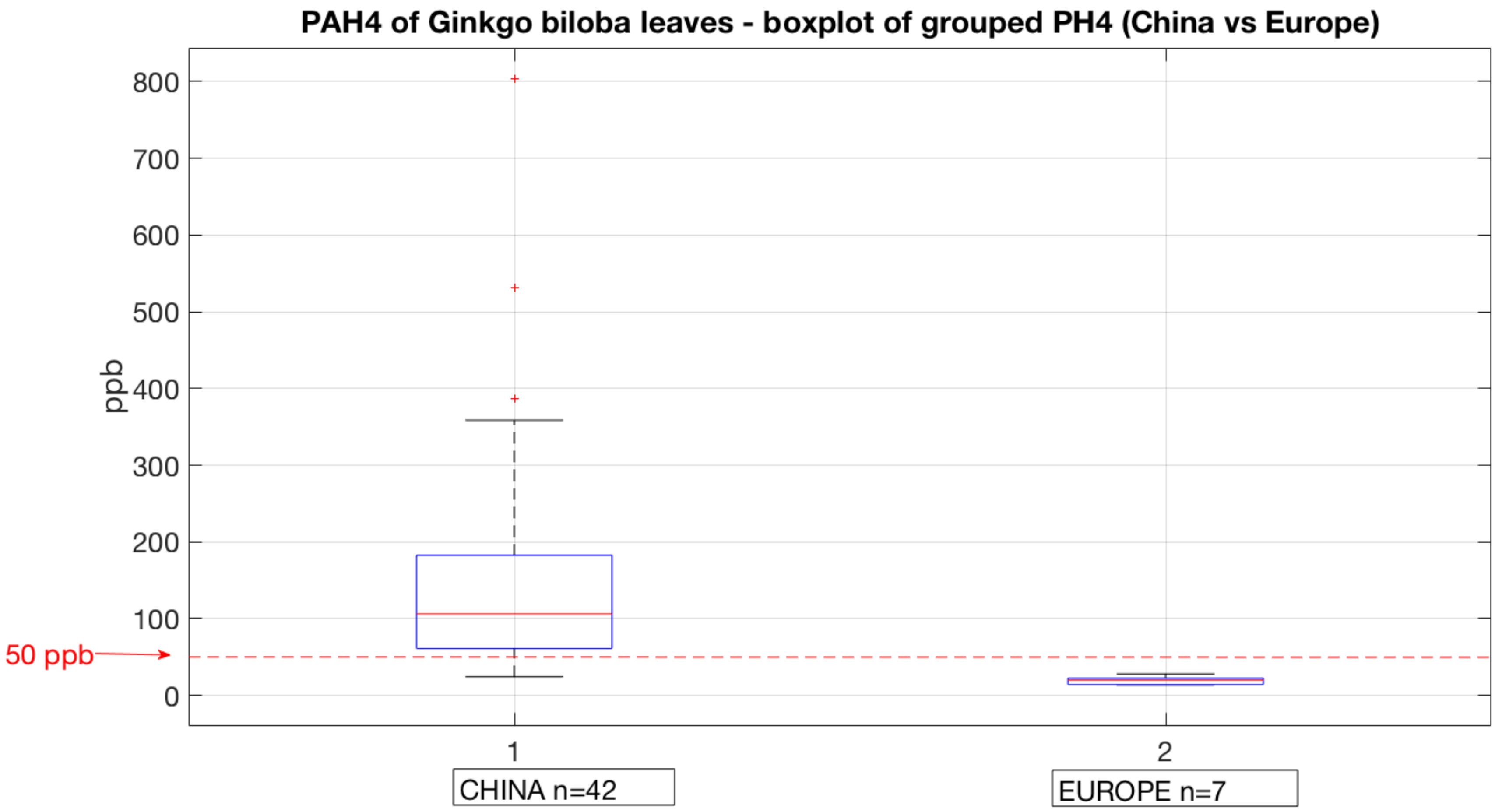
ORIGINS OF THE HERBS: impact on PAH4

Ginkgo biloba leaves from China and Europe

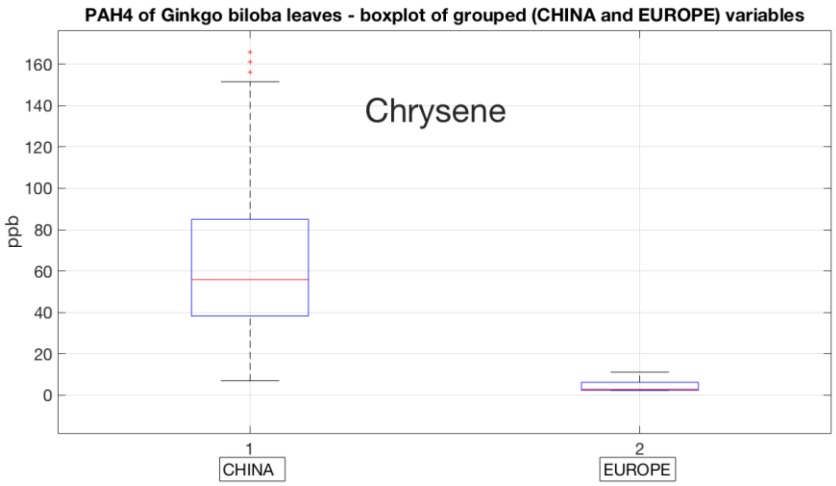
Origin	Samples n.	Drying
China	42 (from 7 Producers)	Direct (coal)
Europe	7 (from 2 Producers)	Indirect

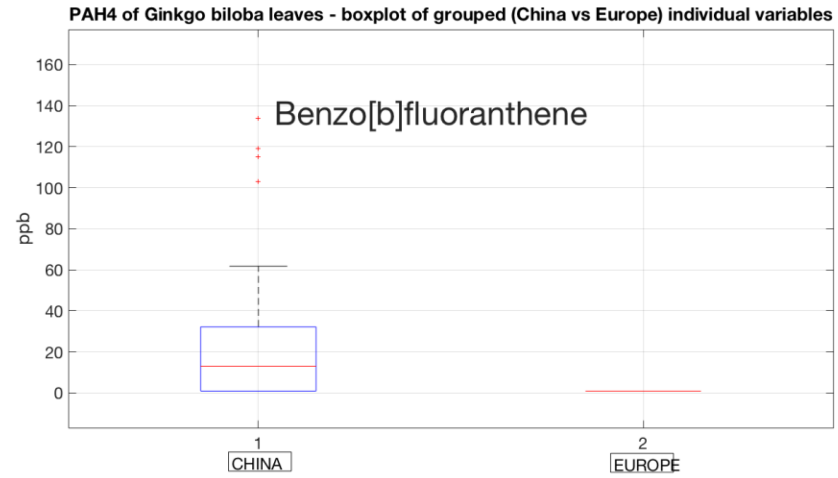
ORIGINS OF THE HERBS: impact on PAH4

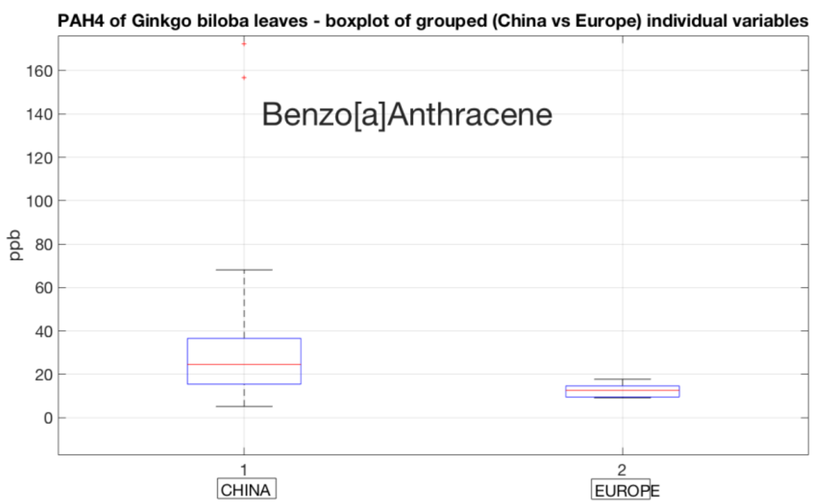
Ginkgo biloba leaves from China and Europe

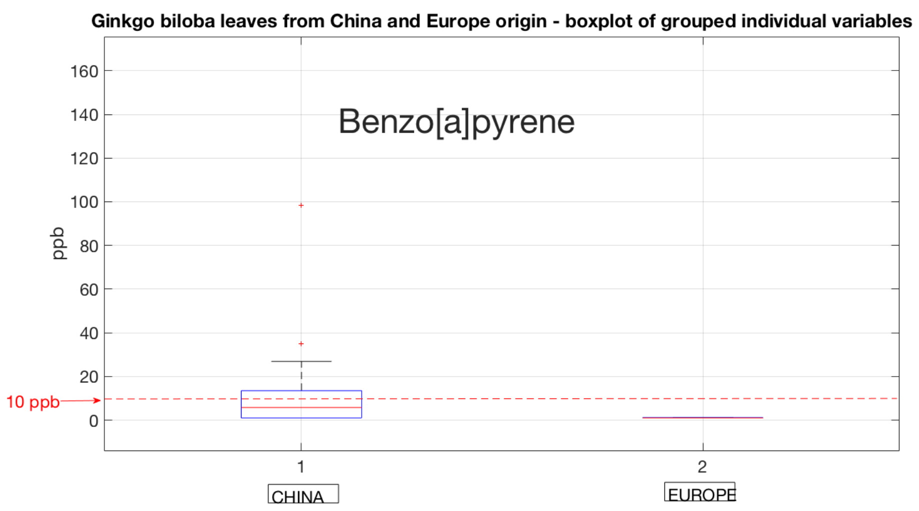


ORIGINS OF THE HERBS: impact on PAH4
Ginkgo biloba leaves from China and Europe









indena®

ORIGINS OF THE HERBS: impact on PAH4

Ginkgo biloba extracts from China and Europe leaves

Samples of extracts	Origin of Starting Herbs	PAH4 (ppb)
39	China and Europe	All absent (< LOQ)

LOQ=1 ppb

ORIGINS OF THE HERBS: impact on PAH4

Ginkgo biloba – Risk evaluation for PAH

HERBS - LEAVES

A **Likely gaseous deposition to leaves via cuticle or via stomata or dry deposition of particles-bound (Europe)**

C **Contamination during drying process: direct drying using coal or wood as combustible (China).**

BOTANICAL EXTRACT

- **Risk elimination** due to lipophilic purification: PAH all purged

PART OF THE PLANT - FRESH FRUITS: impact on PAH4




Name	Samples n.	Content (ppb)
Vaccinum macrocarpon (Cranberry)	9	< 1 (LOQ)
Olea europaea (Olive)	1	< 1 (LOQ)
Vaccinium myrtillus (Bilberry)	3	< 1 (LOQ)
Aristotelia Chilensis (Maqui)	3	< 1 (LOQ)
Total	13	

FRESH FRUITS: impact on PAH4

HERBS - FRESH FRUITS

- Fresh fruits are likely to be **not affected by PAH** due to the limited time to the environmental exposure (see slide «PART OF THE PLANTS»), no contact with soil and no subjected to drying.

SPECIES – *Cynara cardunculus* L.: impact on PAH4

ORIGIN	n. of Samples	Drying	Sum PAH4 (ppb)
Austria	1	Indirect	36
Serbia	1	Indirect	25
Germany	1	Indirect	20
Italy	1	Indirect	15
France	1	Direct (Gas)	< 2.5

indena®

SPECIES – *Cynara cardunculus* L.: impact on PAH4

HERBS - LEAVES

A Likely gaseous deposition to leaves via cuticle or via stomata or dry deposition of particles-bound

B Passive and active uptake soil-to-root (?)

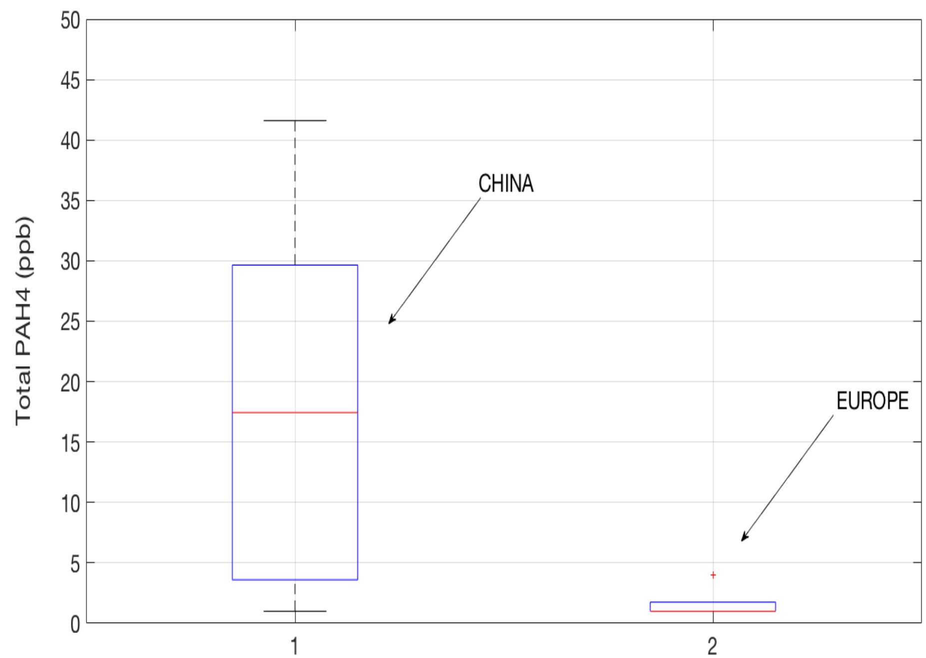
BOTANICAL EXTRACT

- **Risk elimination** due to lipophilic purification: PAH all purged

indena®

SPECIES – *Equisetum arvense:* impact of origin on PAH4

#ID	PAH4 (ppb)	ORIGIN
c41016	20.3	CHINA
c44551	20.2	CHINA
c45994	3.2	CHINA
c47989	41.6	CHINA
c49222	39.0	CHINA
c50908	< 4	CHINA
c51484	< 1	CHINA
c52037	14.7	CHINA
c47197	< 1	BULGARIA
c47715	< 1	BULGARIA
c50239	< 4	BULGARIA
c51795	< 1	ALBANIA
c51796	< 1	ALBANIA



SPECIES – *Equisetum arvense:* impact of origin on PAH4

HERBS - LEAVES

C **Contamination during drying process: direct drying using coal or wood as combustible (China).**

BOTANICAL EXTRACT

- **Risk elimination/riduction:** due to aqueous solvent: PAH not extracted or in minimal amount.

ANALYTICAL METHODS for PAHs

Suitable methods (Sensitivity, Specificity)

1. **GC-MS or GC-MS/MS**
2. HPLC/FLD (High performance liquid chromatography coupled to fluorescence detector)

ANALYTICAL METHODS for PAHs

SAMPLING OF HERBS for PAH4 analysis

- Sampling procedures depend on type and weight of the lot in order to obtain samples that are representative.
- Precautions shall be taken to avoid any change in the composition of the sample and do not became contaminated during sample preparation.
- Containers shall be made of inert material (e.g. glass) and not made of plastic materials like polypropylene or PTFE because they can absorb PAHs.
- Possible losses of PAHs if sample is exposed to light.

Note: Sampling references for benzo[a]pyrene in foodstuff: Commission Regulation No 333/2007

ANALYTICAL METHODS for PAHs

Procedure examples

GC-MS ANALYSIS – STEP 1: REFERENCE SOLUTION

- Reference solution preparation: a mixture of **PAHs certified** purchased from **Ultra Scientific company** (in this mixture the concentration of each PAH is 100 mg/l) and a solution of 7H-benzo[c]fluorene at a concentration of 1000 mg/l;

- At each solution add 10 µl of ISWS solution (mix of **deuterated PAH**) for each ml of reference solution and sample solution (**ISTDs** final concentration 50 µg/l);

- Deuterated PAH4: Benz[a]anthracene-d12, Chrysene-d12, Benzo[b]fluoranthene-d12, Benzo[a]pyrene-d12.

ANALYTICAL METHODS for PAHs

Procedure examples

GC-MS ANALYSIS – STEP 2: TEST SOLUTION

MAY DEPEND ON MATERIAL TYPE:

- HERBAL MATERIALS
- DRY EXTRACTS
- LIPOPHYLIC (OILY) MATERIALS

ANALYTICAL METHODS for PAHs
Procedure examples - HERBS

GC-MS ANALYSIS – STEP 2: TEST SOLUTION PREPARATION

- **Extraction with acetone**;
- **Purification** by gel filtration **after solvent exchange** (from acetone to ethylacetate/cyclohexane 50:50 mixture);
- Analysis by GC/MS technique.

ANALYTICAL METHODS for PAHs

Procedure examples – DRY EXTRACTS

GC-MS ANALYSIS – STEP 2: TEST SOLUTION PREPARATION

- Dissolution of the sample with **water-methanol 50:50 v/v solution**;
- **Liquid-liquid extraction** of PAH4 with **cyclohexane** pesticide grade;
- Analysis by GC/MS technique; if necessary proceed to purification by gel filtration.

ANALYTICAL METHODS for PAHs

Procedure examples – LIPOPHYLIC EXTRACTS

GC-MS ANALYSIS – STEP 2 : TEST SOLUTION PREPARATION

- **Dissolution** of the sample with **petroleum ether or n-hexane**;
- **Liquid-liquid extraction** of PAH4 with **acetonitrile saturated with petroleum ether or n-hexane**;
- Purification by **gel filtration**;
- Analysis by GC/MS technique.

ANALYTICAL METHODS for PAHs

Procedure examples

GC-MS ANALYSIS – STEP 2: ANALYTICAL INSTRUMENTAL CONDITIONS

- *Capillary column type VF-17 ms30 m, i.d.0,25mm, film 0,15 μm or equivalent*
- *Oven:initial temperature 40 °C for 3 min; increase of 10 °C min up to 330 °C; isotherm 4 min, increase of 20 °C min up to 350 °C; isotherm 5 min.*
  Injection temperature: 300 °C.
- *Injection mode: splitless (closing of the valve for 1 minute).*
- *Carrier gas: He (constant column flow 1ml/min)*
- *Injection volume: 1 μl*
- *MassSpectrometer parameters:*
  - *Source Temp: 280 ° C*
  - *GC interface: 280 ° C*
- *Ion source: EI*
- *Electron energy: 70,3 eV*
- *Acquisition Type: SIM*

ANALYTICAL METHODS for PAHs

GC-MS – PROFILE

- GC-MS technique is used, with **single ion monitoring (sim)** acquisition.

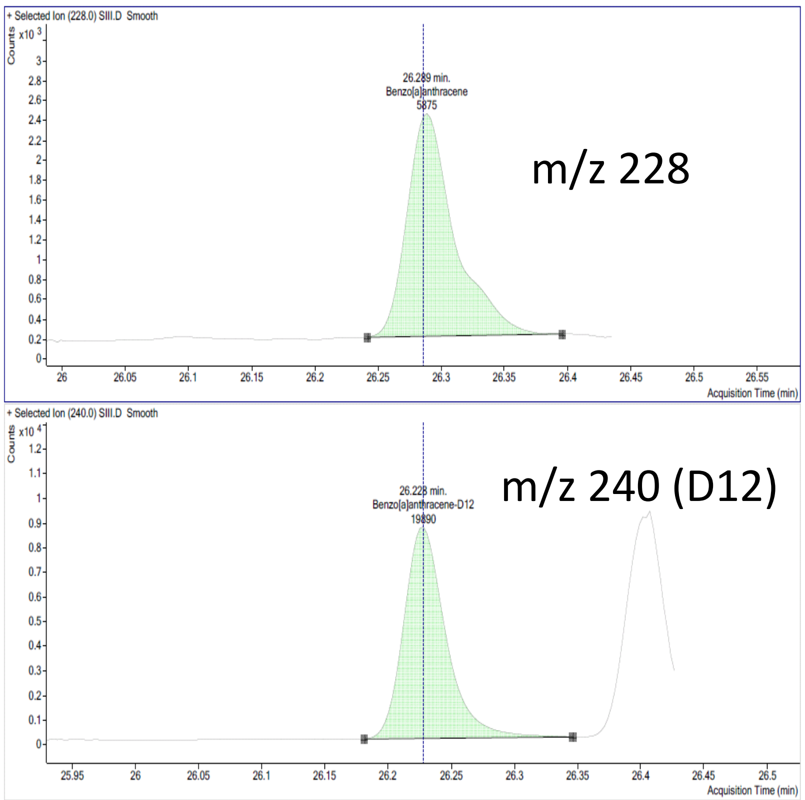
- Due to the nature of the analytes under exam, which present a difficult fragmentation, the use of the technique **GC-MS/MS** does **not lead to significant improvements** in terms of sensitivity and specificity.

- **The quantitation limit** of method is 0.5 ng/ml relative to extraction solution, that corresponds to about **1 ng/g** related to sample for each analyte.

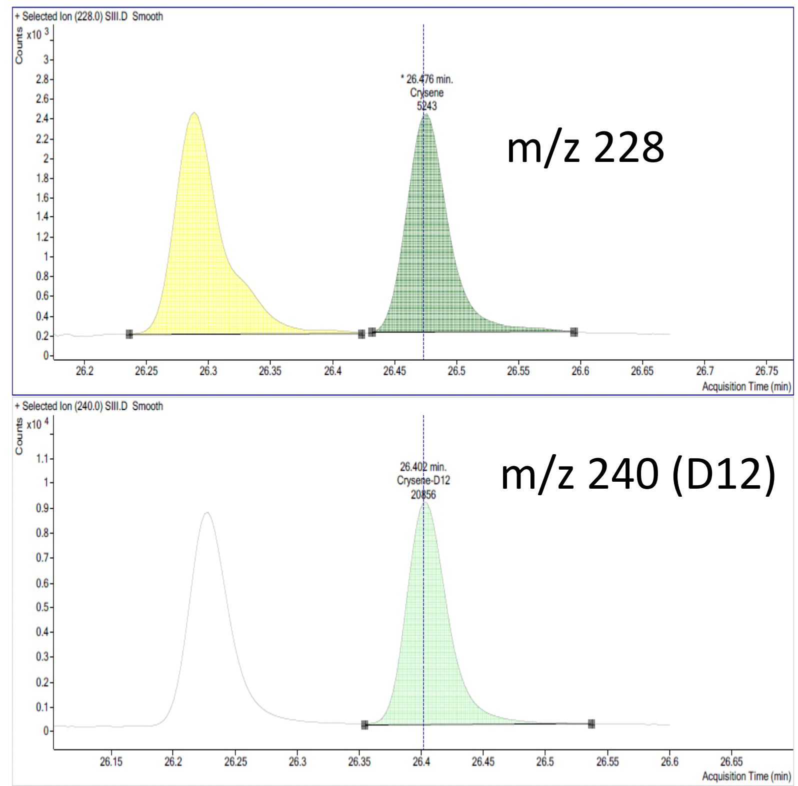
ANALYTICAL METHODS for PAHs

GC-MS – PROFILE

Benzo(a)Anthracene with deuterated iSTD chromatogram



Chrysene with deuterated iSTD chromatogram

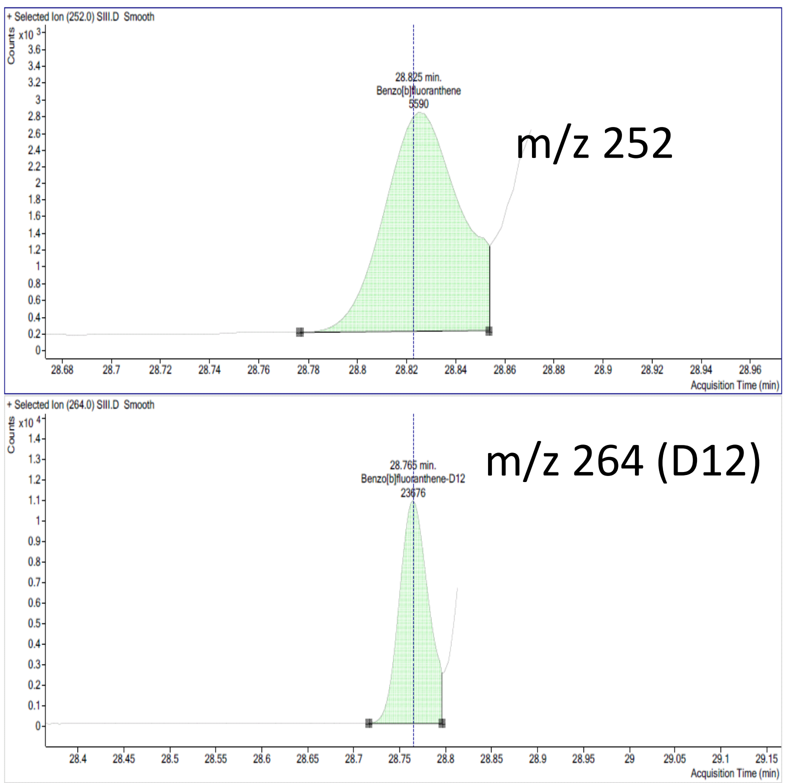


indena®

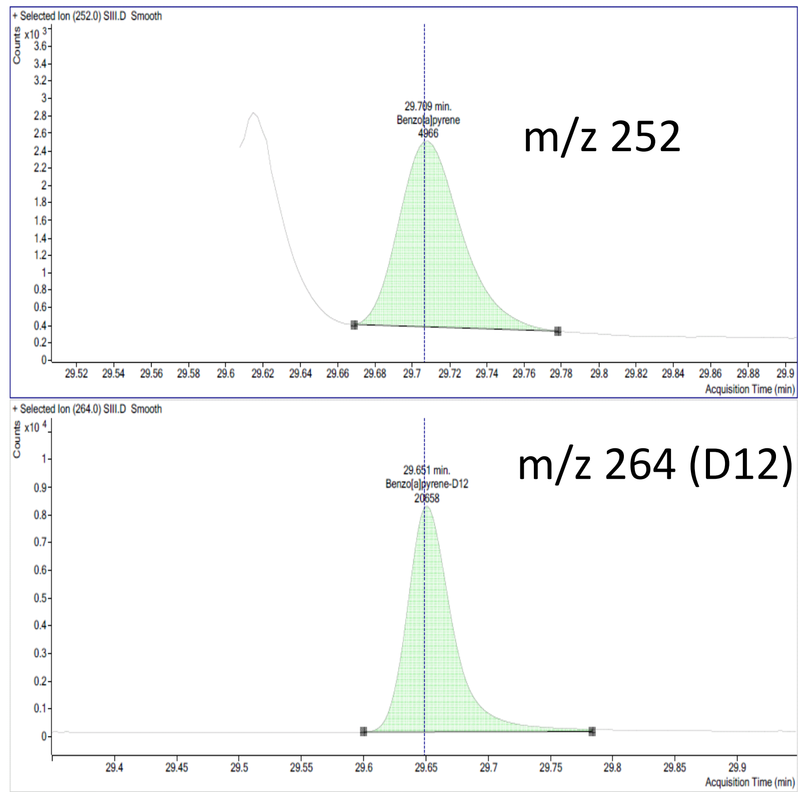
ANALYTICAL METHODS for PAHs

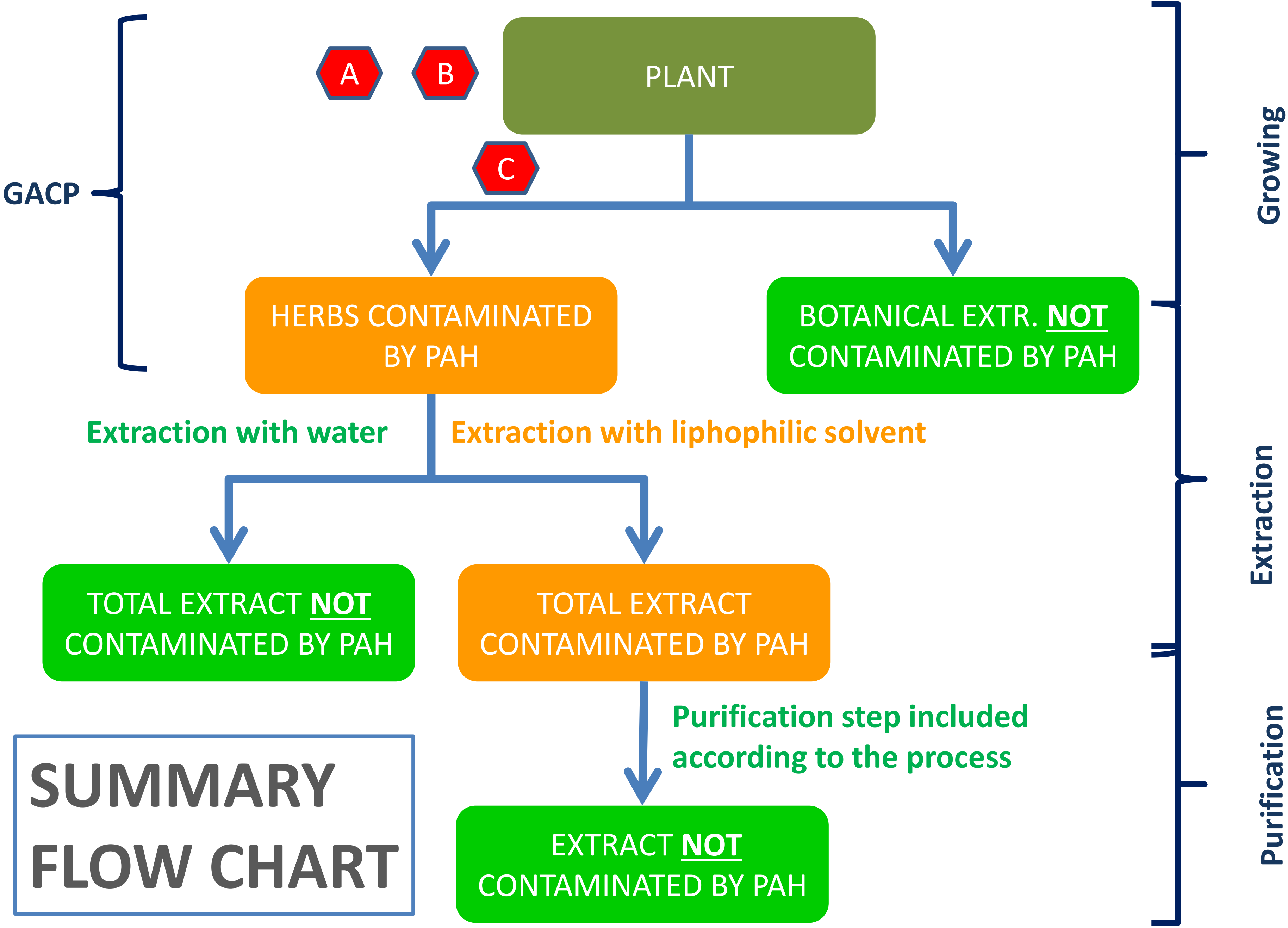
GC-MS – PROFILE

Benzo(b)fluoranthene
with deuterated iSTD chromatogram



Benzo(a)pyrene with
deuterated iSTD chromatogram





GACP
A
B
PLANT
C
HERBS CONTAMINATED BY PAH
BOTANICAL EXTR. **NOT** CONTAMINATED BY PAH
Extraction with water
Extraction with liphophilic solvent
TOTAL EXTRACT **NOT** CONTAMINATED BY PAH
TOTAL EXTRACT CONTAMINATED BY PAH
Purification step included according to the process
EXTRACT **NOT** CONTAMINATED BY PAH
SUMMARY FLOW CHART
Growing
Extraction
Purification

indena®

CONCLUSIONS: GACP MITIGATION

5.1 **Buildings used in the processing** of harvested plants **must be clean….**

7.1 **All processes and procedures** that could affect the quality of the product **must be documented**.

7.3 For cultivated plants/herbal substances **all processing steps** have **to be documented** including the location of cultivation.

7.6 **The geographic location of the collection area** and the harvest period **should be described as** precise as possible.

9.1.1 **Plants should not be grown in soil contaminated** with sludge, heavy metals, residues, plant protection products or other chemicals etc.

CONCLUSIONS: GACP MITIGATION

11.4 Cutting devices or harvesters must be adjusted such that **contamination from soil particles is reduced to a minimum.**

11.5 The harvested plant **should not come into direct contact with the soil**.

12.3 ….**Drying directly on the ground** or under direct exposure to the sunlight **should be avoided** unless specifically required….

13.4 **Packaging materials must be stored in a clean and dry place** …**It must be guaranteed that no contamination** of the product occurs by the use of packaging materials, particularly in the case of fibre bags.

ADDITIONAL ADVICES

- Avoid as much as possible the direct artificial drying of the biomass
- The soil of cultivation should be tested before the cultivation

THANK YOU FOR YOUR KIND ATTENTION

INDENA TODAY

Follow us on:

in YouTube flickr

indena®